VISCOSITY CHANGES IN AGEING UNFERTILIZED EGGS OF ARBACIA PUNCTULATA

A. J. GOLDFORB

With Technical Assistance of Milton Landowne

(*From the College of the City of New York*)

In previous studies it was demonstrated that ageing unfertilized eggs of *Arbacia punctulata* undergo a series of changes including rate of fertilization, membrane formation, and cleavage (Goldforb, 1918*a* and *b*), agglutinin concentration (Goldforb, 1929*a* and *b*), and size (Goldforb, in press). Permeability to water and stretching and bursting of the plasma membrane in hypotonic sea water changed markedly with age. These studies will be reported later. The present study is devoted to a consideration of the viscosity changes.

Procedure

Eggs from each female, if in good physiological condition, were washed in 200 cc. sea water. Aliquot portions were transferred to flat finger bowls with 150 cc. sea water. Morning and evening the supernatant water was removed, the eggs transferred to clean bowls, and sea water added. Sea water was collected in quantity at high tide, filtered and stored. The pH was 8.3. The temperature in the bowls varied from 18 to 22° C., but during an experiment within 2.5°, usually within 1.5° C. At successive ages eggs from the same bowl were centrifuged by a slight modification of the method developed by Heilbrunn (1928).

Constant Centrifugal Force, Varying Time, Early Experiments

In 1930,[1] eggs were centrifuged in 2 mm.-bore tubes in a small electric centrifuge. Immediately after centrifuging, the eggs were examined in sea water. After preliminary trials a period of centrifugation was chosen that gave the following zonation:

(1) Oil cap sharply defined.

(2) Hyaline zone in most eggs extended 45 to 55° [2] from center of oil cap.

(3) Twenty consecutive eggs, with planes of zoning at right angles to the horizontal, were measured.

[1] Dr. V. Schechter assisted in these experiments.

[2] I.e., degrees on circumference from center of oil cap.

(4) Hyaline zone near oil cap free of granules, near grey zone containing numerous scattered granules.

(5) Grey and red zones sharply differentiated.

At successive ages, eggs were centrifuged at the same speed, but the time was varied to approximate the same degree of zonation. Increase in time denoted relative increase in viscosity, and vice versa. The results may be summarized as follows:

Between 10 minutes and 3 hours after shedding, viscosity increased in most experiments. The exceptions will be discussed later.

Between ca. 3 and 30 hours, *viscosity progressively increased with age* in every experiment. For example, in one experiment the time of centrifuging required to approximate the same degree of zonation increased progressively from ¾ to 2½ minutes.

Beginning about 40 hours after shedding, *viscosity progressively decreased.*

This cyclical change in viscosity occurred in all experiments. That the changes were significant was shown by the following: Repetitive tests agreed within 15 seconds; centrifuging time increased with age by 105 or more seconds. Though the temperature during some tests rose to 3½° C., yet when those tests were chosen in which the temperature did not vary beyond 0.5°, the same changes occurred. Though the number of eggs examined at each age was small, yet all experiments gave similar results.

Constant Force and Time, Change in Percentage of Zoned Eggs

In 1931, with the same centrifuge and tubes, the centrifugal force and time were constant at successive ages. The centrifuge registered 4,444 r.p.m. (without load). As the result of preliminary trials a period of centrifugation of 2 minutes duration was usually chosen. This time included $7\frac{1}{2} \pm \frac{1}{2}$ seconds to attain maximal speed, but did not include 26 ± 2 seconds for the centrifuge to stop. The voltage was constant during the day. Tests were made when no other large electrical apparatus was used on the same line. During a test the temperature in the centrifuge head varied between 21.4° and 22.4° C., usually within 0.6°. Immediately after centrifuging, the eggs were transferred to 0.4 per cent formalin (Howard, 1931) and examined within 2 hours. About 100 consecutive eggs, lying at a proper angle, were examined at each age and classified as follows:

Group 1	*Group 2*	*Group 3*
Oil cap very distinct	Same	Less distinct
Hyaline zone, granules few	Many	Very numerous
Hyaline zone, 60–70°	45–60°	Less than 40°
Hyaline grey border, thin straight line	Very ragged	Loose band
Hyaline and grey zones sharply different in color	Less sharp	Intergrades
Oil to grey zone, ca. 15 units	Ca. 12	Less than 10

A typical experiment is summarized in Fig. 1. The eggs of one female were centrifuged for one minute. The number of eggs recorded at each age varied from 84 to 247 (average 194). Between 2¾ and 22 hours after shedding, the adequately zoned (Group 1) eggs *decreased* progressively from 51 to 14 per cent. This decrease denoted correspondingly greater viscosity. After 22 hours, however, there was a reversal. The percentage of Group 1 eggs *increased* from 14 to 51 and then to 84 per cent. This reversal or liquefaction occurred during rapid deterioration.

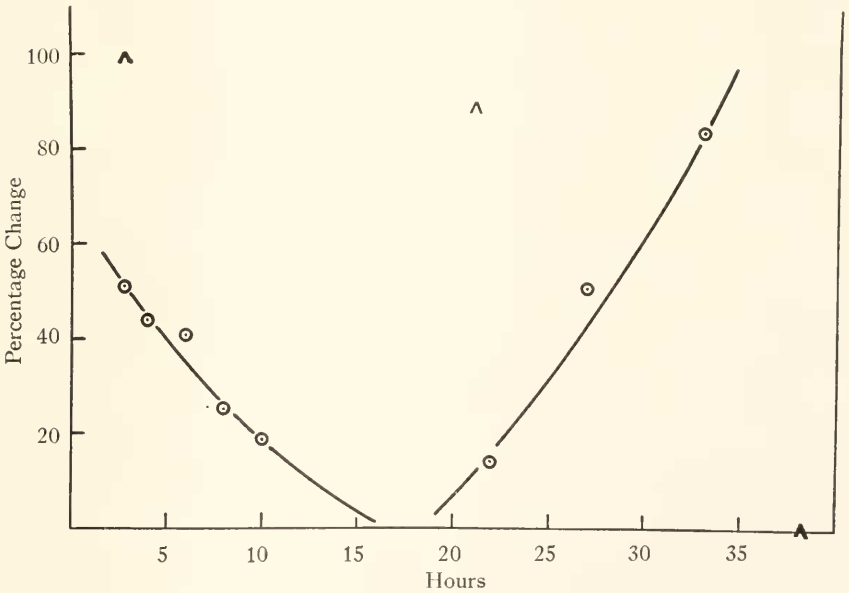

FIG. 1. Centrifuged at 4,444 r.p.m. for 1 minute at 21.5° C. The percentage of eggs zoned to a fixed standard decreased progressively during the first ca. 20 hours and increased thereafter. A decrease in percentage indicates corresponding increase in viscosity and vice versa. ⊙ = per cent zoned; ∧ = per cent cleavage of control eggs.

Similar results were obtained in a second experiment. Eggs 1½ hours old, centrifuged for 2 minutes, gave 85 per cent which were

sharply zoned. Between 1½ and 29 hours the percentage of eggs of Group 1 decreased progressively from 85 to 0 per cent, while the percentage of eggs of Group 2 increased from 5 to 94 per cent. When 48 hours old, Group 2 eggs had decreased from 98 to 38 per cent, while Group 3 (the least zoned) eggs had increased from 6 to 62 per cent. In the case of the older eggs, in order to avoid crushing them at the bottom of the tubes, second samples were centrifuged for one minute only. Between 24 and 48 hours the eggs of Group 2 decreased progressively from 91 to 24 per cent, while Group 3 eggs increased from 9 to 76 per cent.

In a third experiment, the percentage of adequately zoned (Group 1) eggs decreased, between 5 and 48 hours, from 100 to 71 per cent. When centrifuged during late ages, for 1 minute only, the percentage of Group 2 eggs decreased from 85 to 17 per cent.

In the last two experiments liquefaction appears to have begun about the forty-eighth hour. At this age the zonation of many eggs was greater and the hyaline zone contained fewer granules and was much deeper than at any previous age.

It was therefore concluded that viscosity was progressively increased during early and intermediate ages; that there were indications of a progressive liquefaction during late ages; that the degree of change is indicated by the change in percentage of eggs zoned to a fixed standard.

Same Force, Varying Time, Improved Technique

In 1932, viscosity was determined by the following improved technique:

1. *Temperature.*—The centrifuge head was cooled by a regulated flow of ice water. The temperature was recorded at the beginning and end of each test. It varied within 1°, usually within 0.5° C.

2. *Centrifugal Force.*—A powerful Emerson hand centrifuge was used. The time necessary to attain maximum speed was reduced from 7½, in preceding experiments, to 1 second. The time necessary for stopping was reduced from 26 to 1 second.

3. *Low Centrifugal Force.*—After repeated trials a relatively low centrifugal force was selected, viz., 1,750 gravities.

4. The standard of zonation approximated that of Group 2 in previous experiments. At this low centrifugal force and short duration, a small change in centrifuging time gave rise to readily detectable changes in zonation. Hence accuracy of tests was increased.

5. Two or more determinations were made at each age, one usually for the same time as in the accepted previous test, the other for longer

or shorter time. *The objective was to produce not only the same zonation, but the same percentage of similarly zoned eggs.*

6. *The Avoidance of Injury at Bottom of Tube.*—To prevent smashing of eggs at the bottom of the tube, at late ages, a capillary drop of eggs was added to a fixed level of isosmotic solution of C.P. cane sugar solution. The eggs were not injured by the sugar solution, for they cleaved like the control eggs. Nor was their viscosity altered by the solution.

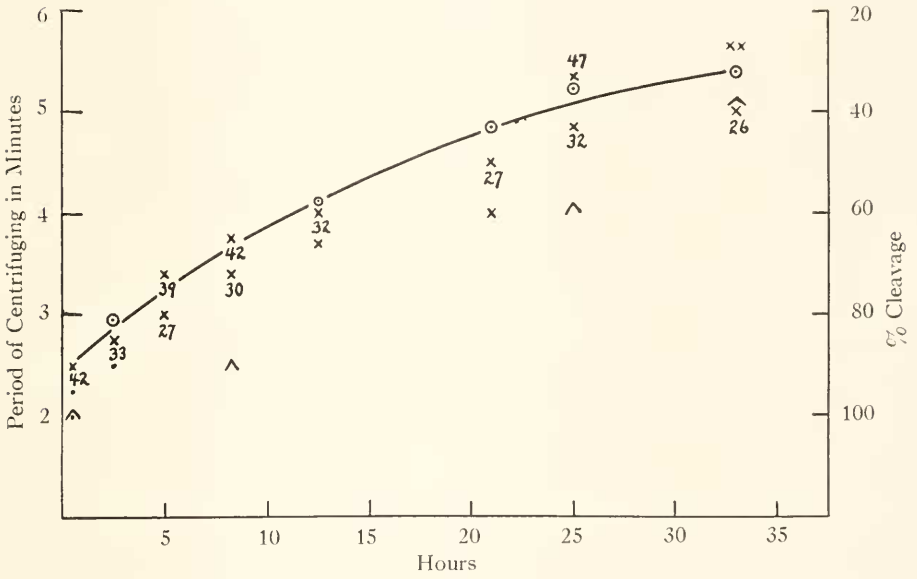

FIG. 2. Centrifuged at 1,750 gravities at 21.5° C. for indicated minutes to approximate same percentage of similarly zoned eggs. Centrifuge time increased during the first 33 hours.

× = observed time in minutes.
⊙ = calculated time.
· = insufficiently zoned.
×× = overzoned.
∧ = percentage cleavage.
Numbers = percentage of eggs zoned to standard.

7. *Sensitivity of Test.* An increase of 10 to 15 seconds longer centrifuging definitely increased the percentage of zoned eggs. For example, in one experiment, eggs centrifuged for 2 minutes resulted in no eggs zoned to standard. With each increase of 10 seconds centrifugation, the percentage of zoned eggs increased to 18, 53, and 86 per cent respectively. In another experiment centrifugation for 1¾ min-

utes produced 34 per cent zoned eggs. Fifteen seconds more centrifugation gave 52 per cent. Repetitive tests varied 0 to 8 per cent.

Cyclical Viscosity, Eggs Centrifuged in Sea Water

In Experiment T_1 (Fig. 2), the first test was made 17 minutes after shedding. When centrifuged for 2 and 2¼ minutes, the eggs were insufficiently zoned. When centrifuged for 2½ minutes, 42 per cent (of 165 eggs) were zoned to a carefully noted standard.

When 2½ hours old, one sample was tested for 2½ minutes as before. The eggs were not sufficiently zoned. The second sample, centri-

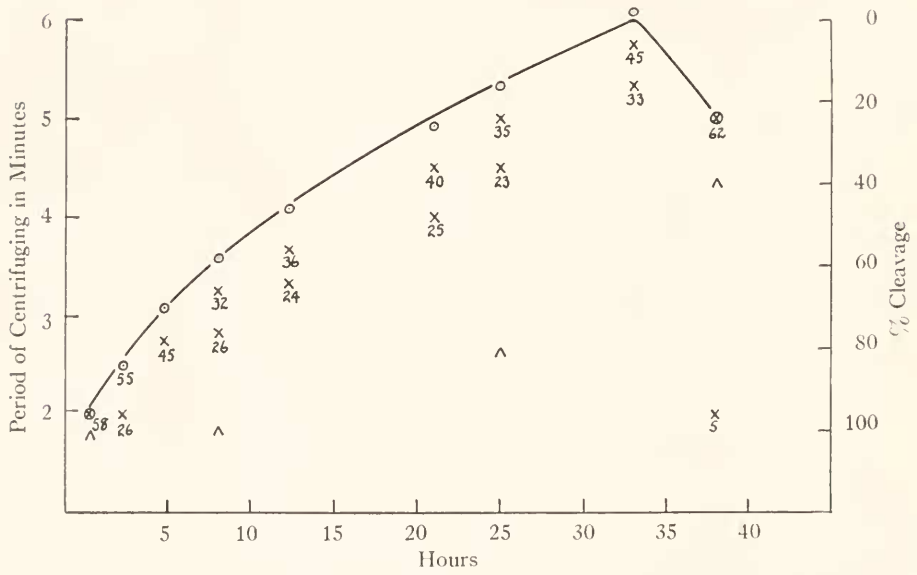

Fig. 3. Same conditions and symbols as in Fig. 2. Shows *increased* viscosity during first 33 hours and decreased viscosity thereafter.

fuged 30 seconds longer, contained only 33 per cent zoned to standard. The estimated time necessary to produce the same percentage of eggs zoned to the same standard as in the initial test was 2′ 55″.

When 5 hours old, 2 samples were again centrifuged, one for 3 minutes (to approximate the previous estimated 2′ 55″) and another for 3′ 25″. The first contained only 27 per cent, the second 39 per cent zoned eggs. The latter approximates the initial 42 per cent. Hence 55 seconds longer centrifuging was required at this age to produce the same zonation as in the ¼-hour old eggs.

Similar double tests were made at successive ages to 33 hours. The

time required to approximate the same percentage of equally zoned eggs increased with age as follows: 2.5, 2.7, 3.4, 3.7, 4.0, 4.5, 5.3, and 5.5 minutes respectively. The estimated time increased progressively from 2½ to 5$^{5}/_{12}$ minutes (Fig. 2). Viscosity increased 116 per cent.

The experiment was repeated with similar results (Fig. 3). Several facts deserve brief mention.

(1) For similar ages the increase in centrifugal time was greater, i.e., from 2 to 6$^{1}/_{12}$ minutes, an increase of 202 per cent.

(2) Liquefaction occurred after 33 hours. Between 33 and 38 hours the centrifugal time decreased from 6½ to 5 minutes. Though

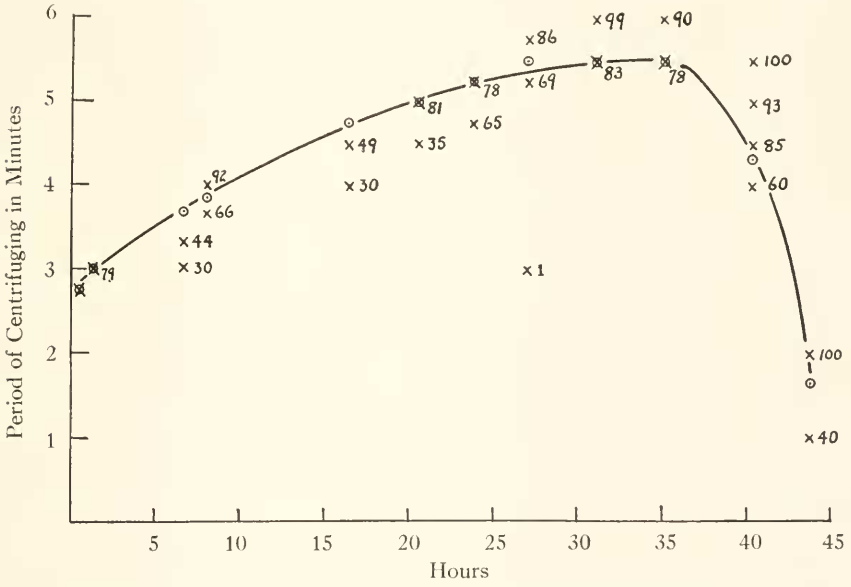

Fig. 4. Centrifuged in isosmotic sugar solution 1,460 gravities. Same symbols as in Figs. 2 and 3. Shows increased viscosity to thirty-fifth hour and progressive decrease to forty-fourth hour.

the eggs were less viscous than at the thirty-third hour, they were much more viscous than at the initial age, for when centrifuged for 2 minutes (the initial time) only 5 instead of 58 per cent of the eggs zoned to standard.

(3) Liquefaction occurred when the eggs were markedly deteriorated.

Cyclical Viscosity, Eggs Centrifuged in Isosmotic Solution

When centrifuged in an isosmotic C.P. cane sugar solution (density 1.085), the eggs were scattered below the sugar level, where the force was 1,460 ± 100 gravities.

In Experiment T_3, for example, 2 to 6 tests were made at each age. An average of 173 eggs were examined after each test. Between 1½ and 35 hours the time of centrifuging, required to produce the same percentage of equally zoned eggs, increased progressively from 2¾ to 5½ minutes (Fig. 4).

When eggs were 27 hours old, it was necessary to centrifuge for 5½ minutes to zone 79 per cent (the initial per cent) to standard. When centrifuged for 3 minutes (the initial duration), only 1 per cent were zoned to standard. This affords another measure of the increased viscosity at this age.

After 35 hours there was rapid liquefaction. When 40½ hours old, a sample centrifuged for 5½ minutes gave 100 per cent either

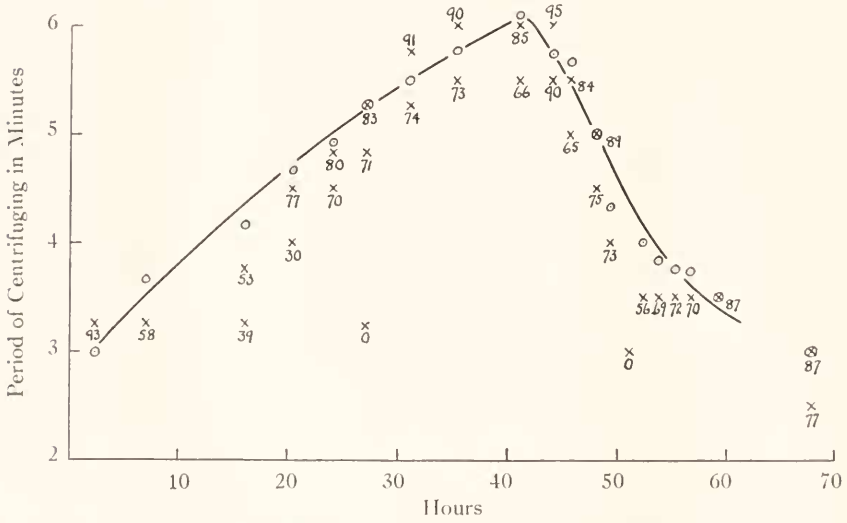

Fig. 5. Another experiment as in Fig. 4, but for more ages. Same cyclical viscosity with age.

zoned to standard or overzoned. Less centrifugation, i.e., 4⅓ minutes, sufficed. When 43½ hours old, eggs were centrifuged for 4½, 4, 3½, 3, and 2 minutes respectively. All these gave 100 per cent zoned or overzoned eggs. The time required to zone to standard had decreased from 5½ to 1⅔ minutes.

In Experiment T_4, Fig. 5, tests were made as in the previous experiment but over a longer series of ages. Viscosity increased progressively during the first 40 hours. The increase was from 3 to 6½ minutes or 100 per cent. Between 44 and 68 hours viscosity decreased rapidly. At late ages, even at the reduced time of centrifugation, nearly all the eggs were overzoned, so that liquefaction was greater than indicated by the figures.

It is therefore concluded that *ageing eggs undergo a cyclical change in viscosity, increasing during the first ca. 35 hours and decreasing thereafter,* and that the increase is of the order of 2 to 3 times the initial viscosity. The subsequent decrease is even greater.

Viscosity of Ripe vs. Unripe Eggs

In the preceding experiments there were a few unripe eggs in nearly every test. None of these, however, showed any trace of zonation. To determine the relative viscosity of ripe and of unripe eggs, females were chosen that contained the largest number of unripe eggs,

Table I

Viscosity of Unripe Eggs

Exp. No.	♀ No.	Age	Centrifugation		Medium	Zoning Unripe Eggs	Zoning Ripe Eggs
			Force	Time			
		hours	*gravities*	*minutes*			
1	1	2	1750	1⅝	Sea Water	0	68% zoned to standard
	2	4	1750	2	" "	0	67 " " "
2	1	1	710	7½	Isosmotic Sugar Sol.	0	30 " " 90°
	1	2	710	10	" " "	0	50 " " "
	1	3	710	15	" " "	0	100 " " 110°
	1	4	710	25	" " "	0	100 " " 120°
3	1	2⅛	1750	1¾	" " "	0	Most " " 45°
	1	1⅝	1750	2	" " "	0	" " " 55°
	1	1¾	4000	1½	" " "	0	" " " 65°
	1	1⅔	4000	2½	" " "	0	" " " 75°
	1	1½	7000	3	" " "	0	" " " 90°
	1	1⅙	7000	5	" " "	0	" " " 110°

and were subjected to longer durations and greater centrifugal forces. Table I summarizes 3 experiments.

Experiment I

Eggs were centrifuged in sea water at 1,750 gravities for 2 minutes. Sixty-eight and 67 per cent of 400 ripe eggs were zoned to standard. None of the unripe eggs in a sample of 1,600 eggs showed any evidence of zonation.

Experiment II

Eggs were centrifuged at 710 gravities for 7½ to 25 minutes. When centrifuged for 25 minutes, all ripe eggs were much overzoned; the hyaline grey border extended from 90 to 120°; yet none of the unripe eggs showed any trace of zonation. A few small unripe eggs formed pseudopodial processes.

Experiment III

Eggs were centrifuged at increasing forces, viz., 1,750, 4,000 and 7,000 gravities for 1¾ to 5 minutes. With each increase in time or force the hyaline zone deepened and the number of scattered granules therein decreased. At the greatest centrifugal force all ripe eggs were not only overzoned but elongated considerably, as described in detail by E. B. Harvey (1932). *Not a single unripe egg was elongated, and none showed any sign of zonation.*

It is therefore concluded that *either the gravimetrically separable substances are not organized in the unripe egg or being present the viscosity is more than eleven times greater than in ripe eggs.*

Unripe eggs vary in size to a very much greater degree than ripe eggs, yet no difference in viscosity was detectable between the very small (young), and the large (older) unripe eggs. These observations suggest that, with maturation, there is not only a very profound but possibly a very rapid liquefaction. Liquefaction appears to be far greater than during polar body formation or cleavage, or following changes of temperature, salts, etc. (Heilbrunn, 1921, 1924, 1928).

Discussion

It was shown above that in the life history of the *Arbacia* egg there were five viscosity phases, viz.:

1. *Extreme Viscosity, Unripe Eggs.*—Whatever their age (or size) all unripe eggs were extremely viscous. None could be zoned by the considerable forces and durations used. That unripe eggs were more viscous than ripe ones was noted by Paspaleff (1927) and by Heilbrunn (1928). Our study corroborates these findings and supplies a quantitative measure of the difference, namely that unripe eggs are at least 11 times more viscous than freshly-shed ripe eggs.

2. *Liquefaction.*—Upon maturation there was a very marked and probably very rapid liquefaction in all eggs.

3. *Increasing Viscosity, Ripe eggs, Early Ages.*—All ripe eggs progressively increased in viscosity with age. The rate of increase was slower when the eggs were aged within the body, faster when they were aged in sea water. Viscosity increased during the first ca. 35 (22 to 41) hours after shedding. The increase was 2 to 3-fold.

4. *Decreasing Viscosity, Ripe Eggs, Late Ages.*—After ca. 35 hours there was a reversal, namely, a progressive liquefaction. The rate was faster and the total change was greater than during earlier ages.

5. *Extreme Viscosity, Death.* Our evidence is not conclusive whether at the greatest ages death was directly accompanied by coagula-

tion or whether extreme liquefaction occurred first, followed quickly by coagulation.

These five phases are diagrammatically represented in Fig. 6.

Extent of Change.—These viscosity changes are greater than those which occur under a variety of other conditions, but comparisons are not readily made, either because the changes have not been given quantitatively or because different units have been used. In Table II such a comparison is attempted. This table shows that viscosity in-

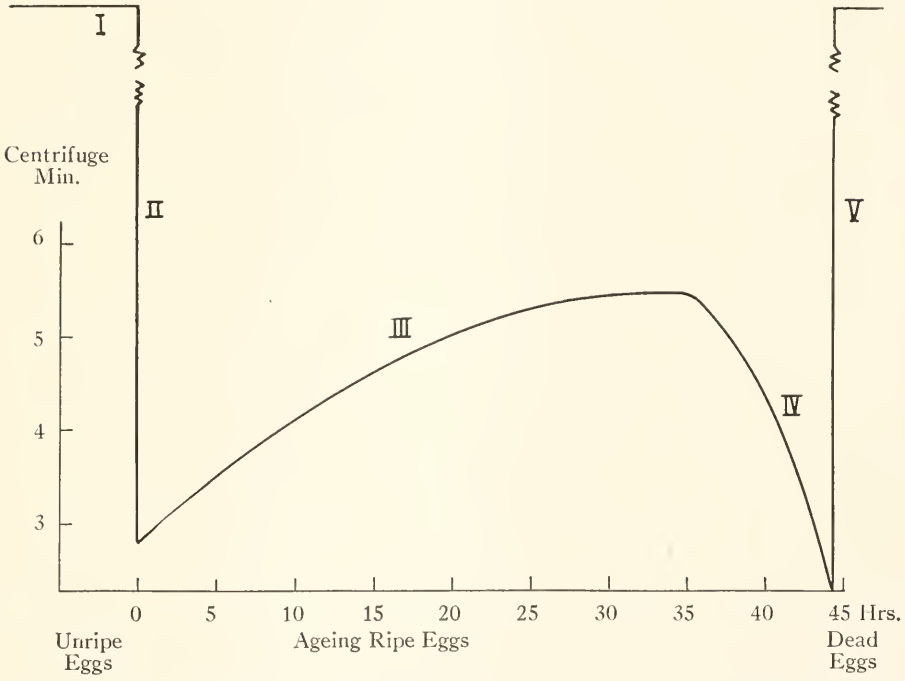

FIG. 6. Diagrammatic representation of the five phases in viscosity of the egg, viz.

I. All unripe eggs at any age, extremely viscous.
II. Maturation, extreme and rapid liquefaction.
III. Ripe eggs, early and intermediate ages, progressively increased viscosity.
IV. Ripe eggs, late ages, much deterioration, progressive liquefaction.
V. Dead eggs, coagulation.

creased during mitosis of *Arbacia* eggs by two-thirds. Larger changes occurred with temperature, HCl, NH_4OH, ultraviolet light, etc., viz., 2¼ to 4 x. The largest change heretofore described occurred during mitosis of *Cumingia eggs*, viz., 5 to 8x. During ageing of *Arbacia* eggs, there were both positive and negative changes of 2 to 11x.

The factors which play a minimal or no rôle in these changes include body fluid, temperature, and pH.

Modification of Egg by Body Fluid.—Body fluid decreases cleavage (Lillie, F. R., 1923; Ephrussi, 1925). Boguchi (1930) believed that this was due to intestinal, not to body fluid. Body fluid alters the reaction of eggs to calcium (Heilbrunn, 1925, 1928). In a later study it will be shown that body fluid does decrease cleavage, gives rise to irregular cleavage, accelerates swelling in hypotonic solutions, and increases viscosity. In these experiments body (perivisceral) fluid was withdrawn by pipette through the oral disc, centrifuged, and the supernatant fluid used. The freshly-shed "dry" eggs or eggs from removed

TABLE II

Comparison of Viscosity Changes under Various Conditions

Author	Inducing Conditions	Cell	Viscosity Change
Heilbrunn, 1920	Mitosis	Arbacia Eggs	+ 2/3
Pantin, 1924	Temperature −0.7 to 30° C.	Nereis "	− 3 2/5
Heilbrunn, 1921	" 2 to 15° C.	Cumingia "	+ 2 1/4
" "	" 15 to 30° C.	" "	− 2 1/4
Barth, 1929	CO_2, pH 8.3 to 5.0	Arbacia "	+ 2 1/2
	HCl " 8.3 to 3.0	" "	+ 2 1/2
	NH_4OH " 8.1 to 11.0	" "	+ 4
Heilbrunn and Young, 1930	Ultra-violet ray	" "	+ 3 to 4
Heilbrunn, 1921	Mitosis	Cumingia "	+ 5 to 8
Goldforb	Unripe eggs	Arbacia "	+11
	Ripe eggs, early ages	" "	+ 2 to 3
	" " , late "	" "	− 3 to 4
	Dead "	" "	+11

ovaries were immediately transferred to this fluid. At successive ages one sample of eggs washed in sea water and another unwashed sample were centrifuged simultaneously in sea water. The body fluid eggs were in every experiment more viscous than eggs from which the body fluid had been washed away. The difference was small but definite. By ca. the sixth hour, viscosity was the same in both kinds of eggs. Therefore neither the marked liquefaction upon maturation, nor the striking increase in viscosity with age may be attributed to the effect of body fluid. It was pointed out above that in a few of the early experiments there was no rise in viscosity during the first 5 hours. This we believe was due to a comparison of eggs whose body fluid had not been thoroughly washed away (hence with greater viscosity) with eggs at the next age when washing had been complete.

Temperature and Viscosity.—In *Nereis* eggs a rise in temperature from —0.7 to + 30° C. decreased viscosity 3½ times (Pantin, 1924). In *Cumingia* eggs a similar increase in temperature gave rise to a cyclical change in viscosity with maxima at 0, 15, and 32° C. (Heilbrunn, 1924). In our experiments there were five phases with maximal changes of 11 times, but the variation in temperature was within 2.25° C. When those tests were selected during which the temperature had not changed more than 1 or 0.5° C., the same viscosity changes occurred. Temperature, therefore, did not give rise to the marked viscosity changes in ageing eggs.

Hydrogen Ion Concentration.—Acid tends to increase, alkali to decrease viscosity (Jacobs, 1922; Barth, 1929); but viscosity begins to change in acid medium below pH 7.8 (Barth, 1929). In our experiments the transfer from body fluid to sea water decreased viscosity slightly. This was probably due to the change from the acid body fluid, viz., pH 7.4, to alkali sea water, viz., pH 8.3. With ageing, the pH of the supernatent sea water decreased slowly but not below 7.9. Hence this change in hydrogen ion concentration *per se* is not a significant factor in the large viscosity changes during ageing. During latest ages, liquefaction may possibly be due in part to the rapid and considerable inflow of sea water rendering the interior less acid.

Granule Size.—Increased viscosity may be due to the formation of new granules or the increased size of old granules (Heilbrunn, 1928). We were unable, as were previous workers, to demonstrate such changes. Conversely the decreased viscosity during latest ages may be due to diminution of the granules in one or more layers. The four zones[3] were present at all ages, but their volumes changed during latest ages. The oil zone appeared to be unchanged. The hyaline zone, however, was much larger, in extreme instances two or more times the size at previous ages, when the same forces and durations were used. This zone was entirely free of grey granules. More significant is the diminution of both grey and red zones. They shrank from a previous ca. 120°[4] to about ⅓ or 45° in depth. This may mean either a diminution in size of the same number of granules or a loss in number of granules or both. Either supposition may account for the liquefaction at these late ages, but due to this change in depth of zones, an exact comparison of the viscosity at these late ages with the viscosity at earlier ages may not therefore be made.

Ion Penetration.—Rate of ion penetration may change with age. Osterhout (1926) and Brooks (1929) found that K accumulated in the

[3] The fifth zone, described by E. B. Harvey (1932), was not studied.
[4] Measured in degrees on the periphery.

cell sap of ageing *Valonia*. Stiles and Kidd (1919), studying the relative penetration of K, Ca, Na, and Mg in ageing carrot and potato cells, discovered that calcium changed most with age. The effect of changing concentration of ions with age requires further study. The calcium change is now under investigation.

Permeability.—Viscosity and water intake both increased during mitosis and cell division (Heilbrunn, 1920*a*; Haberlandt, 1919, 1920). In our next study it will be shown that permeability to water progressively and markedly increased in ageing *Arbacia* eggs. Increased viscosity in these eggs occurred with increasing permeability to water. Both increased during the first ca. 35 hours after shedding. Thereafter permeability increased much further, while viscosity was reversed. Similar reversal of viscosity occurred with increasing CO_2 (Jacobs, 1922), increasing alkalinity (Barth, 1929), increasing temperature (Heilbrunn, 1920*b*, 1924). It is extremely interesting that Dhar (1930) obtained a reversal of viscosity in ageing hydrophyllic colloids. In our experiments the liquefaction at late ages may be due in part to excess water, to decreased acidity of interior of egg, or to excess calcium.

Injury.—Without attempting to define injury, it may be said that increasing permeability and viscosity occurred with little or no protoplasmic deterioration. Deterioration was measured by decreased and by irregular cleavage. In Experiment T_2, Fig. 3, for example, between ¼ and 8 hours, the viscosity increased 79 per cent, i.e., the centrifugal time necessary to zone to standard increased from 120 to 215 seconds. During these ages cleavage was normal and decreased but 1 per cent (i.e., from 100 to 99). Between 8 and 33 hours viscosity increased to 204 per cent. Irregular cleavage began at the twenty-first hour. Cleavage decreased at the thirty-third hour to 80 per cent. Viscosity, during these ages, increased with progressive injury. Buenning (1926) had observed this phase only. During later ages there was considerable and rapid deterioration. This was associated with decreased viscosity.

Summary

1. Relative viscosity was determined by (*a*) change in percentage of eggs zoned to a fixed standard, at constant centrifugal force and time; (*b*) change in time at a given force to produce same percentage of equally zoned eggs.

2. Ageing unfertilized eggs manifested five major phases in viscosity, viz.

(*a*) Unripe eggs. No trace of zoning could be induced. They were at least eleven times more viscous than mature eggs.

(*b*) All mature eggs were readily zoned at much lower forces and duration. Maturation is accompanied by profound and apparently rapid liquefaction.

(*c*) During the first ca. 35 hours after shedding, ripe eggs progressively *increased* in viscosity, 2 to 3 ×. At constant force and time, the zoned eggs decreased with age from 85 to 0 per cent. To produce the same percentage of equally zoned eggs, the centrifuging time increased from ca. 2 to 5 minutes. Similar results were obtained when eggs were centrifuged in isosmotic sugar solution.

(*d*) With further ageing, viscosity was reversed. There was progressive liquefaction. The decrease was 3 to 4 ×.

(*e*) Upon death viscosity was again sharply increased at least eleven times.

3. The amount of change with age is greater than that during mitosis, or induced by heat, acid, light, etc.

4. Temperature and acid are excluded as factors in the cyclical viscosity changes with age.

5. Perivisceral fluid is also not responsible.

6. Increasing permeability to water is associated with increasing viscosity during early and intermediate ages. During late ages, with excess water intake, viscosity is reversed.

7. Viscosity increased during early ages with no detectable injury. It increased further with progressive injury, i.e., during intermediate ages. It decreased during the period of most rapid injury.

BIBLIOGRAPHY

Barth, L. G., 1929. *Protoplasma,* **7**: 505.
Boguchi, M., 1930. *Protoplasma,* **11**: 432.
Buenning, E., 1926. *Bot. Arch.,* **14**: 138.
Brooks, S. C., 1929. *Protoplasma,* **8**: 389.
Dhar, N. R., 1930. *Jour. Phys. Chem.,* **34**: 549.
Ephrussi, B., 1925. *C. R. Acad. Sci.,* **180**: 775.
Goldforb, A. J., 1918*a*. *Biol. Bull.,* **34**: 372.
Goldforb, A. J., 1918*b*. *Biol. Bull.,* **35**: 1.
Goldforb, A. J., 1929*a*. *Biol. Bull.,* **57**: 333.
Goldforb, A. J., 1929*b*. *Biol. Bull.,* **57**: 350.
Goldforb, A. J., in press.
Haberlandt, G., 1919. *Sitzungsber. d. k. Preus. Akad. d. Wiss.,* Berlin, pp. 322 and 721; 1920, p. 323.
Harvey, E. B., 1932. *Biol. Bull.,* **62**: 155.
Heilbrunn, L. V., 1920*a*. *Jour. Exper. Zoöl.,* **30**: 211.
Heilbrunn, L. V., 1920*b*. *Biol. Bull.,* **39**: 307.
Heilbrunn, L. V., 1921. *Jour. Exper. Zoöl.,* **34**: 417.
Heilbrunn, L. V., 1924. *Am. Jour. Physiol.,* **68**: 645.
Heilbrunn, L. V., 1925. *Science,* **61**: 236.
Heilbrunn, L. V., 1927. *Arch. Exp. Zellf.,* **4**: 246.
Heilbrunn, L. V., 1928. The Colloid Chemistry of Protoplasm, Gebruder Borntraeger, Berlin.

Heilbrunn, L. V., and R. A. Young, 1930. *Physiol. Zool.*, 3: 330.
Howard, E., 1931. *Biol. Bull.*, 60: 132.
Jacobs, M. H., 1922. *Biol. Bull.*, 42: 14.
Lillie, F. R., 1923. Problems of Fertilization, Chicago, p. 172.
Lillie, R. S., 1909. *Am. Jour. Physiol.*, 24: 14.
Lillie, R. S., 1911. *Am. Jour. Physiol.*, 28: 197.
Lillie, R. S., 1912. *Am. Jour. Physiol.*, 29: 372.
Lillie, R. S., 1913. *Am. Jour. Physiol.*, 31: 255.
Osterhout, W. J. V., 1926. *Proc. Soc. Exper. Biol. and Med.*, 24: 234.
Pantin, C. F. A., 1924. *Jour. Marine Biol. Ass.*, 13: 331.
Paspaleff, G., 1927. *Publ. d. Stazione Zool. d. Napoli*, 8: 1.
Stiles, W., and F. Kidd, 1919. *Proc. Royal Soc.*, B., 90: 487.